

## PATENT COOPERATION TREATY

PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

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|   |   |
|---|---|
| <b>Date of mailing (day/month/year)</b><br>09 July 1999 (09.07.99)              |   |
| <b>International application No.</b><br>PCT/US98/21895                          | <b>Applicant's or agent's file reference</b><br>09010/030WO1        |
| <b>International filing date (day/month/year)</b><br>15 October 1998 (15.10.98) | <b>Priority date (day/month/year)</b><br>15 October 1997 (15.10.97) |
| <b>Applicant</b><br>SHORT, Jay, M.  |   |

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:

14 May 1999 (14.05.99)

☐ in a notice effecting later election filed with the International Bureau on:
2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

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|--|---|
| <p>The International Bureau of WIPO<br/>34, chemin des Colombettes<br/>1211 Geneva 20, Switzerland</p> <p>Facsimile No.: (41-22) 740.14.35</p> | <p>Authorized officer<br/>D. Barmes</p> <p>Telephone No.: (41-22) 338.83.38</p> |
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

|   |           |  |
|---|-----------|--|
| <b>(51) International Patent Classification <sup>6</sup> :</b><br><b>C12Q 1/68, G01N 33/53</b>  | <b>A1</b> | <b>(11) International Publication Number:</b> <b>WO 99/19518</b><br><b>(43) International Publication Date:</b> 22 April 1999 (22.04.99)   |
| <b>(21) International Application Number:</b> PCT/US98/21895<br><b>(22) International Filing Date:</b> 15 October 1998 (15.10.98)<br><br><b>(30) Priority Data:</b><br>60/062,073 15 October 1997 (15.10.97) US<br><br><b>(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application</b><br>US 60/062,073 (CIP)<br>Filed on 15 October 1997 (15.10.97)<br><br><b>(71) Applicant (for all designated States except US):</b> DIVERSA, INC. [US/US]; 10665 Sorrento Valley Road, San Diego, CA 92121 (US).<br><br><b>(72) Inventor; and</b><br><b>(75) Inventor/Applicant (for US only):</b> SHORT, Jay, M. [US/US]; 320 Delage Drive, Encinitas, CA 92024 (US).<br><br><b>(74) Agent:</b> HAILE, Lisa, A.; Fish & Richardson P.C., Suite 1400, 4225 Executive Drive, La Jolla, CA 92037 (US).  |           | <b>(81) Designated States:</b> AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).<br><br><b>Published</b><br><i>With international search report.</i> |
| <b>(54) Title:</b> SCREENING FOR NOVEL COMPOUNDS WHICH REGULATE BIOLOGICAL INTERACTIONS<br><br><b>(57) Abstract</b><br><br>Disclosed is a process for identifying compounds having a specified activity of interest, which process comprises (i) introducing interacting molecules into a host cell under conditions to generate or repress a detectable signal; and (ii) introducing a third compound or gene or genes encoding a third compound into the host cell from (i); and (iii) screening said host cell utilizing a method for detecting the inhibition or enhancement of interaction of proteins or other molecules in an in vivo or in vitro system. Another aspect of the present invention provides a process for identifying compounds of interest, which process comprises (i) generating one or more expression libraries derived from nucleic acid directly isolated from the environment; and (ii) screening said libraries utilizing a method for detecting the inhibition or enhancement of interaction of proteins or other molecules in an in vivo or in vitro system. |           |  |

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Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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| EE | Estonia                  |    |  |    |  |    |                          |

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/21895

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12Q 1/68; G01N 33/53  
US CL : 435/6, 7.1

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
|-----------|--|-----------------------|
| X         | US 5,525,490 A (ERICKSON et al.) 11 June 1996, entire document, especially columns 15-16.  | 1-8                   |
| X         | MENDELSON et al. Applications of interaction traps/two-hybrid systems to biotechnology research. Curr. Opin. Biotechnol. October 1994, Vol. 5, pages 482-486, especially page 485.                       | 1-4                   |
| X         | CHIU et al. RAPT1, a mammalian homolog of yeast Tor, interacts with the FKBP12/rapamycin complex. Proc. Natl. Acad. Sci. USA. 20 December 1994, Vol. 91, No. 26, pages 12574-12578, see entire document. | 1, 3, 4, 8, 9         |

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

|   |  |
|---|--|
| * Special categories of cited documents:  | *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention  |
| *A* document defining the general state of the art which is not considered to be of particular relevance  | *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone   |
| *E* earlier document published on or after the international filing date  | *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | *G* document member of the same patent family  |
| *O* document referring to an oral disclosure, use, exhibition or other means  |  |
| *P* document published prior to the international filing date but later than the priority date claimed  |  |

Date of the actual completion of the international search

14 DECEMBER 1998

Date of mailing of the international search report

27 JAN 1999

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Authorized officer

ROBERT SCHWARTZMAN

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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/21895

| C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT |  |                       |
|---|--|-----------------------|
| Category*   | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
| X   | CHAUDHURI et al. The interaction between the catalytic A subunit of calcineurin and its autoinhibitory domain, in the yeast two-hybrid system, is disrupted by cyclosporin A and FK506. FEBS Lett. 03 January 1995, Vol. 357, No. 2, pages 221-226, especially page 224. | 1, 2, 4, 8, 9         |
| X   | YANG et al. Cyclophilin A and FKBP12 interact with YY1 and alter its transcriptional activity. J. Biol. Chem. 23 June 1995, Vol. 270, No. 25, pages 15187-15193, especially page 15189.  | 1, 2, 4, 8, 9         |

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/21895

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

STN: Medline Biosis Embase CAPlus WPIDS

APS

Search Terms: two hybrid, interaction trap, polyketide, FK506, tetracycline, erythromycin, daunomycin

# PATENT COOPERATION TREATY

## PCT

### INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

REC'D 07 MAR 2000

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|  |  |   |
|--|--|---|
| Applicant's or agent's file reference<br>09010/030WO1  | FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |   |
| International application No.<br>PCT/US98/21895  | International filing date (day/month/year)<br>15 OCTOBER 1998  | Priority date (day/month/year)<br>15 OCTOBER 1997 |
| International Patent Classification (IPC) or national classification and IPC<br>IPC(7): C12Q 1/68; G01N 33/53 and US Cl.: 435/6, 7.1 |  |   |
| Applicant<br>DIVERSA CORPORATION   |  |   |

- This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
- This REPORT consists of a total of 4 sheets.  
☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).  
 These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

|  |  |
|--|--|
| Date of submission of the demand<br><br>14 MAY 1999  | Date of completion of this report<br><br>08 FEBRUARY 2000                        |
| Name and mailing address of the IPEA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br><br>Facsimile No. (703) 305-3230 | Authorized officer<br><br>ROBERT SCHWARTZMAN<br><br>Telephone No. (703) 308-0196 |

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US98/21895

## I. Basis of the report

1. This report has been drawn on the basis of *(Substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments)*:

☒ the international application as originally filed.

☒ the description, pages 1-58, as originally filed.

pages NONE, filed with the demand.

pages NONE, filed with the letter of \_\_\_\_\_.

pages \_\_\_\_\_, filed with the letter of \_\_\_\_\_.

☒ the claims, Nos. 1-15, as originally filed.

Nos. NONE, as amended under Article 19.

Nos. NONE, filed with the demand.

Nos. NONE, filed with the letter of \_\_\_\_\_.

Nos. \_\_\_\_\_, filed with the letter of \_\_\_\_\_.

☒ the drawings, sheets/fig 1-6, as originally filed.

sheets/fig NONE, filed with the demand.

sheets/fig NONE, filed with the letter of \_\_\_\_\_.

sheets/fig \_\_\_\_\_, filed with the letter of \_\_\_\_\_.

2. The amendments have resulted in the cancellation of:

☒ the description, pages NONE.

☒ the claims, Nos. NONE.

☒ the drawings, sheets/fig NONE.

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box Additional observations below (Rule 70.2(c)).

4. Additional observations, if necessary:

NONE



# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US98/21895

## V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

### 1. STATEMENT

|                               |        |              |     |
|-------------------------------|--------|--------------|-----|
| Novelty (N)                   | Claims | <u>10-15</u> | YES |
|                               | Claims | <u>1-9</u>   | NO  |
| Inventive Step (IS)           | Claims | <u>10-15</u> | YES |
|                               | Claims | <u>1-9</u>   | NO  |
| Industrial Applicability (IA) | Claims | <u>1-15</u>  | YES |
|                               | Claims | <u>NONE</u>  | NO  |

### 2. CITATIONS AND EXPLANATIONS

Claims 1-8 lack novelty under PCT Article 33(2) as being anticipated by Erickson et al.

Erickson et al. teaches a modified yeast two-hybrid assay to detect molecules that inhibit protein-protein interactions (column 15, line 28-column 16, line 62). The assay involves a yeast host cell comprising a first interacting protein linked to a DNA binding moiety and a second interacting protein linked to a transcriptional activation moiety. The molecule to be tested is introduced to the hybrid proteins and the interaction of the two hybrid proteins monitored by measuring expression of a reporter gene whose transcription is controlled by the complex of the two hybrid proteins. The DNA binding moiety and the transcriptional activation moiety can be derived from the same transcriptional activator or from different transcriptional activators (column 5, lines 31-45). The detectable protein can be beta-galactosidase (column 6, lines 25-31). The test molecule can be a protein encoded by a polynucleotide which is part of an expression vector (column 16, lines 5-13).

Claims 1-8 lack novelty under PCT Article 33(2) as being anticipated by Mendelsohn et al.

Mendelsohn et al. teaches various forms of the yeast two hybrid assay in which the interaction of a first test protein linked to a DNA binding moiety and a second test protein linked to a transcriptional activation moiety is monitored using a reporter gene responsive to the interaction of the two test proteins. In one version the assay is used to screen for compounds that regulate protein-protein interactions by contacting a cell comprising the two hybrid proteins with the test compound (page 485, column 1, paragraph 2). The DNA binding moiety and the transcriptional activation moiety can be derived from the same transcriptional activator or from different transcriptional activators. The detectable protein can be beta-galactosidase or green fluorescent protein. The test molecule can be a protein encoded by a polynucleotide which is part of an expression vector. (Continued on Supplemental Sheet.)

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US98/21895

## Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Sheet 10

Continuation of: Boxes I - VIII

### V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):

Claims 1, 3, 4, 8 and 9 lack novelty under PCT Article 33(2) as being anticipated by Chiu et al.

Chiu et al. teaches (see entire document) a yeast two hybrid assay comprising a first test protein (FKBP12) linked to a DNA binding moiety (LexA), a second test protein (calcineurin A or an expression library) linked to a transcriptional activation moiety (VP16) and a reporter gene (beta-galactosidase). The test proteins were screened for their ability to interact in the presence of the polyketide rapamycin.

Claims 1, 2, 4, 8 and 9 lack novelty under PCT Article 33(2) as being anticipated by Chaudhuri et al.

Chaudhuri et al. teaches (see entire document) a yeast two hybrid assay comprising a first test protein (calcineurin A) linked to a DNA binding moiety (ACE1), a second test protein (calcineurin A autoinhibitory domain) linked to a transcriptional activation moiety (ACE1) and a reporter gene (beta-galactosidase). The test proteins were screened for their ability to interact in the presence of the polyketide FK506.

Claims 1, 3, 4, 8 and 9 lack novelty under PCT Article 33(2) as being anticipated by Yang et al.

Yang et al. teaches (see entire document) a yeast two hybrid assay comprising a first test protein (FKBP12) linked to a DNA binding moiety (Gal4), a second test protein (YY1) linked to a transcriptional activation moiety (Gal4) and a reporter gene (beta-galactosidase). The test proteins were screened for their ability to interact in the presence of the polyketide FK506.

Claims 10-15 meet the criteria set out in PCT Article 33(2)-(3) because the prior art does not teach or fairly suggest the claimed invention.

Claims 1-15 meet the criteria set out in PCT Article 33(4) for industrial applicability.

NEW CITATIONS

NONE

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

|   |  |   |   |
|---|--|---|---|
| (51) International Patent Classification <sup>6</sup> :<br><b>C12Q 1/68, G01N 33/53</b>   |  | <b>A1</b>   | (11) International Publication Number: <b>WO 99/19518</b>     |
|   |  |   | (43) International Publication Date: 22 April 1999 (22.04.99) |
| (21) International Application Number: PCT/US98/21895   |  | (81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). |   |
| (22) International Filing Date: 15 October 1998 (15.10.98)  |  |   |   |
| (30) Priority Data:<br>60/062,073 15 October 1997 (15.10.97) US   |  | Published<br>With international search report.  |   |
| (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application<br>US 60/062,073 (CIP)<br>Filed on 15 October 1997 (15.10.97) |  |   |   |
| (71) Applicant (for all designated States except US): DIVERSA, INC. [US/US]; 10665 Sorrento Valley Road, San Diego, CA 92121 (US).                    |  |   |   |
| (72) Inventor; and<br>(75) Inventor/Applicant (for US only): SHORT, Jay, M. [US/US]; 320 Delage Drive, Encinitas, CA 92024 (US).                      |  |   |   |
| (74) Agent: HAILE, Lisa, A.; Fish & Richardson P.C., Suite 1400, 4225 Executive Drive, La Jolla, CA 92037 (US).                                       |  |   |   |

~~(54) Title: SCREENING FOR NOVEL COMPOUNDS WHICH REGULATE BIOLOGICAL INTERACTIONS~~

~~(57) Abstract~~

Disclosed is a process for identifying compounds having a specified activity of interest, which process comprises (i) introducing interacting molecules into a host cell under conditions to generate or repress a detectable signal; and (ii) introducing a third compound or gene or genes encoding a third compound into the host cell from (i); and (iii) screening said host cell utilizing a method for detecting the inhibition or enhancement of interaction of proteins or other molecules in an in vivo or in vitro system. Another aspect of the present invention provides a process for identifying compounds of interest, which process comprises (i) generating one or more expression libraries derived from nucleic acid directly isolated from the environment; and (ii) screening said libraries utilizing a method for detecting the inhibition or enhancement of interaction of proteins or other molecules in an in vivo or in vitro system.